THE FERMENTATION OF ALPHA-METHYLGLUCOSIDE BY BACTERIA[1]

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Koser and Saunders (1932) found that *a*-methylglucoside was not fermented by typical cultures of *Bacterium coli*, while members of the Aerogenes group usually attacked it with the production of acid and gas. They reported positive fermentation also by the majority of the methyl-red positive, Voges-Proskauer negative, citrate and cellobiose positive "coli-aerogenes intermediate" types isolated from soil. Later (1933), these workers showed that comparatively few bacteria ferment this carbohydrate; in particular, of the 24 species studied, *Bacterium aerogenes*, *Bacterium Friedländeri* and *Proteus vulgaris* were the only active fermenters, while streptococci from scarlet fever, septic sore throat and erysipelas and the pneumococci decomposed it slowly. These findings suggested that the fermentation of *a*-methylglucoside might serve as a convenient additional taxonomic characteristic, particularly in the case of the Escherichia-Aerobacter group. Accordingly, the ability to ferment *a*-methylglucoside has been determined for a large number of bacterial species, belonging in many genera. The results are presented in this report.

METHODS AND MATERIALS

A total of 730 cultures, representing approximately 60 species or groups, was employed. This number includes 472 Escherichia-Aerobacter strains isolated from human urine and feces and from

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water. The rest were selected from the laboratory collection. The characteristics of most of the cultures were determined in detail to permit exact taxonomic allocation. An exception was made, however, for the sporogenous cultures, in which case the species designations listed are those given by the laboratories from which the cultures were obtained.

The test medium was prepared by adding *a*-methylglucoside (Eastman), to yield a concentration of 0.5 per cent, to nutrient broth, composed of 0.3 per cent meat extract (Bacto) and 0.5 per cent peptone (Bacto), which had been adjusted to pH 7.2. Bromcresol purple served as an indicator. The broth was dispensed in the Durham type of fermentation tube, and sterilized by autoclaving for twenty minutes at 15 pounds pressure.

Incubation was carried out at 37°C. Observations were made for two weeks, unless both acid and gas were produced sooner. The results were recorded daily during the first week, and every second or third day during the second.

RESULTS

The results of the tests for *a*-methylglucoside fermentation by all of the genera and species studied are recorded in table 1. Relatively few were able to utilize this carbohydrate although all, with the exception of *Alcaligenes faecalis*, fermented glucose. All of the Aerobacter cultures, regardless of species, decomposed the glucoside with the production of acid and gas, but none of the typical members of the Escherichia group were able to do so. Many of the Escherichia-Aerobacter "intermediate" strains produced acid and gas, but the majority of them failed to utilize the carbohydrate. The Proteus cultures differed significantly in their ability to attack the glucoside. Proteus X19 and X2, and one unclassified strain produced acid only, while *Proteus mirabilis*, Proteus XK and 16 unclassified strains failed to show evidence of fermentation. *Bacillus subtilis* (Ford) was the only culture of the spore-forming group which fermented *a*-methylglucoside. Because of this exceptional behavior, it was replated several times and subcultures isolated. These sub-cultures behaved exactly like the parent culture. The behavior of *Gaffkya tetra-*

TABLE 1

Alpha-methylglucoside fermentation

ORGANISMS	NUMBER OF STRAINS	FERMENTATION	ORGANISMS	NUMBER OF STRAINS	FERMENTATION
Aerobacter aerogenes	34	AG	Shigella paradysenteriae (Sonne)	19	
Aerobacter oxytocum	27	AG	Shigella dispar	11	
Aerobacter cloacae	16	AG	Shigella alkalescens	2	
Aerobacter levans	2	AG	Shigella ambigua	2	
Aerobacter unclassified	21	AG	Pseudomonas "pyocyaneus"	4	
Escherichia-Aerobacter "intermediates"	20	AG	Flavobacterium vitarumen	1	
Escherichia-Aerobacter "intermediates"	49		Flavobacterium unclassified	1	A
Escherichia "coli" group	208		Chromobacterium violaceum	1	
Escherichia "communior" group	95		Serratia unclassified	7	
Alcaligenes faecalis	3		Acetobacter unclassified	1	
Proteus mirabilis	2		Bacillus cereus	1	
Proteus X19 (Felix)	2	A	Bacillus megatherium	1	
Proteus X19	2	A	Bacillus mycoides	1	
Proteus XK (Kingsbury)	2		Bacillus niger	1	
Proteus X2 (Felix)	2	A	Bacillus subtilis (Koch)	1	
Proteus unclassified	16		Bacillus subtilis (Marburg)	1	
Proteus unclassified	1	A	Bacillus subtilis (Soule)	1	
Salmonella suipestifer	3		Bacillus subtilis (Ford)	1	A
Salmonella aertrycke	4		Bacillus pseudotetani	1	
Salmonella enteriditis	19		Bacillus anthracis	1	
Salmonella Schottmülleri	8		Bacillus unclassified	5	
Salmonella paratyphi	8		Staphylococcus aureus	13	
Salmonella gallinarum	18		Staphylococcus albus	4	
Salmonella pullorum	36		Neisseria catarrhalis	2	
Salmonella Morgani	4		Sarcina citrea	1	
Hirshfeld's bacillus	1		Sarcina ureae	1	
Voldagsen's bacillus	1		Rhodococcus rhodochrous	2	
Eberthella typhi	13		Gaffkya tetragena	1	A*
Shigella dysenteriae (Shiga)	3		Micrococcus aurantiacus	1	A*
Shigella paradysenteriae (Flexner)	8		Micrococci unclassified	12	
Shigella paradysenteriae (Hiss and Russell)	1				
Total number of strains				730	

AG = acid and gas; A = acid only.

* Delayed fermentation.

gena and *Micrococcus aurantiacus* was similar to that of certain streptococci studied by Koser and Saunders (1933). A definite acidity was not produced until the second week of incubation.

The relation of *a*-methylglucoside fermentation to the common taxonomic characteristics of the Escherichia-Aerobacter cultures, and to their sources, is shown in table 2. The Aerobacter and "typical" Escherichia strains showed perfect correlation with all of these differential tests. In the Escherichia-Aerobacter "intermediate" group, however, the only reciprocal relationship found was that between the methyl-red and the Voges-Proskauer reactions. Of the 69 cultures tested, 61 fermented cellobiose; 43 utilized citrate and 20 fermented *a*-methylglucoside. Thus, a better correlation was shown between the fermentation of *a*-methylglucoside and the Voges-Proskauer reaction, than between either the fermentation of cellobiose or the utilization of citrate and the Voges-Proskauer reaction. No connection was found between *a*-methylglucoside fermentation and the source of the cultures; in fact, no reciprocal relationship was observed

TABLE 2

Correlation of alpha-methylglucoside fermentation with other differential tests of the Escherichia-Aerobacter cultures and with their sources

GROUP	SOURCE	NUMBER OF CULTURES	METHYL RED TEST		VOGES-PROSKAUER TEST		UTILIZATION OF CITRATE		FERMENTATION OF CELLOBIOSE		FERMENTATION OF *a*-METHYL-GLUCOSIDE	
			+	−	+	−	+	−	+	−	+	−
Aerobacter	Urine	27	0	27	27	0	27	0	27	0	27	0
	Feces	20	0	20	20	0	20	0	20	0	20	0
	Water	53	0	53	53	0	53	0	53	0	53	0
Escherichia	Urine	190	190	0	0	190	0	190	0	190	0	190
	Feces	90	90	0	0	90	0	90	0	90	0	90
	Water	23	23	0	0	23	0	23	0	23	0	23
Escherichia-Aerobacter "intermediates"	Urine	24	24	0	0	24	9	15	19	5	0	24
	Feces	16	16	0	0	16	12	4	15	1	7	9
	Water	20	20	0	0	20	13	7	18	2	6	14
	Soil	9	9	0	0	9	9	0	9	0	7	2
Total		472	372	100	100	372	143	329	161	311	120	352

between any of the differential characteristics and the source of the bacteria.

DISCUSSION

It is evident that the substitution of a methyl group for the hydrogen of the hydroxyl group attached to the number one carbon atom of glucose markedly influences the availability of the carbohydrate for bacteria, as reported by Kendall and Gross (1930) and by Koser and Saunders (1933).

The results obtained with the Escherichia-Aerobacter group agree with those of Koser and Saunders (1932) and Poe (1934). They are also in accord with the findings of Kendall and Gross (1930) for Escherichia cultures and for *Aerobacter cloacae,* but not for *Aerobacter aerogenes.* These workers, using a single strain, reported that "*B. lactis-aerogenes*" failed to ferment *a*-methylglucoside. It is evident from the observations of Hees and Tropp (1926), Koser and Saunders (1932) and ourselves, however, that *Aerob. aerogenes* ferments *a*-methylglucoside.

In the cases of the Aerobacter and typical Escherichia strains, the fermentation of *a*-methylglucoside correlates perfectly with the production of acetyl-methyl-carbinol, the methyl-red reaction, the utilization of citrate and the fermentation of cellobiose (table 2). For the separation of these two groups, therefore, the fermentation of *a*-methylglucoside can be employed as well as any other of the reactions. On the other hand, this characteristic cannot be used alone to differentiate the Escherichia-Aerobacter "intermediate" cultures from both Aerobacter and typical Escherichia strains. It may be usefully employed, nevertheless, as a means of distinguishing species or types within this "intermediate" group. It is significant that in the utilization of *a*-methylglucoside most of the "intermediate" strains are similar to typical Escherichia.

The findings show that neither the fermentation of *a*-methylglucoside nor any other characteristic, alone or in combination, will indicate whether or not the source of Escherichia-Aerobacter strains is fecal or non-fecal. Furthermore, they emphasize the fact that Aerobacter and Escherichia-Aerobacter "intermediate" cultures may be found in human excreta.

The results obtained with the Proteus cultures (table 1) indicate that the fermentation of *a*-methylglucoside may be used advantageously for distinguishing certain closely related species or types. Our cultures of *Proteus mirabilis* failed to attack the glucoside, while Kendall and Gross and also Koser and Saunders (1933) reported that *Proteus vulgaris* fermented it. Proteus XK also failed to ferment *a*-methylglucoside, while the closely related Proteus X strains decomposed it.

SUMMARY

1. The ability to ferment *a*-methylglucoside has been determined for 730 bacterial cultures representing 19 genera and more than 60 species.

2. Few species were able to attack the glucoside.

3. All of the Aerobacter cultures and approximately one-third of the Escherichia-Aerobacter "intermediate" strains produced acid and gas, while none of the "typical" Escherichia showed evidence of any fermentative power.

4. The relation of *a*-methylglucoside fermentation to other differential characteristics of the Escherichia-Aerobacter group, and to their sources, is recorded.

5. The fermentation of *a*-methylglucoside will differentiate Aerobacter from "typical" Escherichia strains and can be employed advantageously for distinguishing species or types within the Escherichia-Aerobacter "intermediate" group. On the contrary, it will not differentiate "intermediate" cultures from both Aerobacter and "typical" Escherichia strains or indicate whether or not the source of Escherichia-Aerobacter cultures is fecal or non-fecal.

6. The value of *a*-methylglucoside fermentation as an additional aid in the separation of certain closely related Proteus cultures is indicated.

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